SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1. FACS analysis of HIV retroviral restriction by A3G variants.

Shown are the FACS analyses of infections that occurs at the highest viral titer examined (50 ng p24, infecting 10^5 Jurkat cells). All the viruses are NL4-3 Δ Vif- Δ E-eGFP variants which were generated with the encapsidated A3G variant or no A3G as indicated.

Supplemental Figure 2. A3G loop variants mutagenize HIV.

The likelihood of a G→A transition mutation at each position in a region of the HIV *pol* gene was computed for hypermutated viral genomes recovered from infection with virus containing A3G variants. Numbering is relative to the reference strain HXB2 (HIV Sequence Locator, Los Alamos National Laboratories).

Supplemental Figure 3. Expression of AID variants.

A, Expression of AID variants in DT40 B-cells. AID constructs were stably transfected into AID--UNG--IgM+ DT40 cells. After 8-14 weeks in culture, IgM- DT40 cells were collected from pooled transfectants and RNA was prepared. Real-time PCR analysis of mRNA expression of AID is shown, relative to β-actin expression. Results shown are from three replicates with associated standard deviation. N.D., not detected.

B, Expression of AID variants in mouse AID-/- splenic B cells. Constructs were introduced by retroviral infection into AID-/- mouse splenic B cells. Quantiative PCR analysis of mRNA expression is shown, relative to β -actin expression showing similar expression levels of AID variants. Error bars represent the standard deviation from at least three measurements. N.D., not detected.

Supplemental Figure 4. Impact of local targeting on SHM and CSR is proportionally related to *in vitro* deamination levels.

A, Mistargeting to non-CDR regions is proportional to non-hotspot targeting. In vitro data was derived from studies on AID181-WT, AID181-3FL and AID181-3GL previously reported (Kohli et al., 2009; Ref 27). The average rate of targeting AID hotspots (WRC) was calculated from the reported deamination of S60-AAC + S60-AGC + S60-TAC + S60-TGC. The rate of deamination of non-WRC sequences is calculated from the average rate of deamination of all other S60-XXC substrates. Plotted is the ratio of WRC to non-WRC deamination on the left y-axis (red). The right y-axis shows CDR to non-CDR targeting taken from Table 2.

B, Switching is proportional to targeting of the AID hotspot WRC. In vitro data was derived from the studies as above, with the average deamination of S60-WRC substrates on the left y-axis (red). The right y-axis reproduces the amount of class switching to IgG1 observed in Fig. 4.

Supplemental Figure 5. Selection of loop grafting site in AID influences resulting activity.

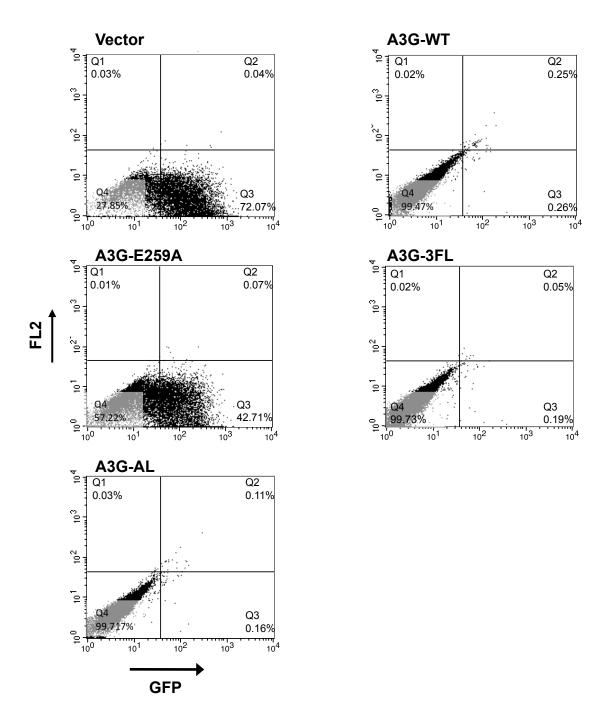
A, Alignment of loop graft variants evaluated. Our initial evaluation of loop graft variants (Kohli et al., 2009; Ref 23) grafted the entire region from 113-123 into the scaffold of maltose binding protein fused, C-terminally truncated AID (AID181-WT). The selection of the loop origin was based on the recognition of conserved flanking sequences, to generate the variant AID181-3GL. In other studies (Wang et al., 2010; Ref 25), for A3G loop graft variants were evaluated that started at position 115. To increase activity "upmutant" version of AID had to be used which containing an additional three mutations that increased catalytic activity. A version of the AID181-3GL variant was created to mimic this shifted position grafting position (AID181-3GL I113L). To further evaluate this position an additional variant AID181-3GL I113A was evaluated. B, Assay for shift to A3G specificity. Single stranded 27-mer DNA substrates with a central CCC motif (S27-CCC) and a 3'-fluorescein residue (FAM) were used in the assay. The substrates contained no additional cytidine residues in the rest of the sequence. 1 μM S27-CCC substrate

was incubated with 1 μ M AID variant and 1 U UNG (NEB) in 20 mM Tris-Cl (pH 8.0), 30 mM NaCl, 1 mM DTT, 5 mM EDTA for 12 hrs at 30 °C, followed by heating to 95 °C for 20 min. Abasic sites were cleaved by the addition of an equal volume of formamide and 1/10 volume of 2 M NaOH followed by incubation at 95 °C for 20 min. Samples were run on a 20% acrylamide/TBE/urea gel and imaged using a Typhoon variable mode imager.

C, Identity of position 113 impacts catalytic activity. Assay with the AID181 variants demonstrates that AID181-WT shown limited deamination of the A3G hotspot CCC. By contrast AID181-3GL is highly proficient at deamination of S27-CCC. The identity of the position 113 residue is significant, as I113A variant shown negligible activity and I113L is compromised for deamination. We therefore conclude that the need to evaluate "upmutant" versions of AID could have resulted from the inappropriate selection of the initial position for loop grafting.

Table S1: Oligonucleotide substrates, primers, probes and mutagenic oligonucleotides

A3G-pCDNA Forward A3G-pCDNA Reverse A3G-pCDNA Reverse A3G-STF. Forward A3G-STF. Forward A3G-STF. Forward A3G-STF. Forward A3G-STF. Reverse BCG CCG CTG CTG TAT TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc cac ctg CTG TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc acc ctg CTG TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc acc ctg cac A3G-AL Forward CTG TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc CTG TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc CTG TAT TTT TGG GAC ACT GAC TAC cag gag ggg ctg cac ctg gc CTG TAC TTT TGG GAC AAT ACT ACG AGC TTC GAC GAC GAC GAG GCG GAC GAG GAG GAC AGG GAG GA		T
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AID-RV Forward AID-RV Reverse CCC CTA CCC GGT AGA ATT CGC GGC CGC AID-RV-3FL Forward AID-RV-3FL Forward AID-RV-3FL Reverse CTG GTA GTC AGT GTC CCA AAA ATA gta gag gcg ctg cgg gtg cac AID-RV-3FL Reverse CTG GTA GTC AGT GTC CCA AAA ATA gta gag gcg ccg ggt gaa gat cct AID-RV-3GL Forward ATT TAC GAC GAC CAA GGG AGG TGC CAG gag ggg ctg cgg gtg cac AID-RV-3GL Reverse CTG GCA CCT CCC TTG GTC GTC GTA AAT gcg cgc ggt gaa gat cct cag act AID-RV-88A Forward AAC GGC TGC CAC GTG GCA CTG CTC TTC CTC CGC AID-RV-E58A Reverse GCG GAG GAA GAG CAG TGC CAC GTG GCA CTG CTC TGC CTC AID-RV-E58A Reverse GCG GAG GAA GAG CAG TGC CAC GTG GCA GCC GTT AID-RV Empty FOR AAG AAG AGA AGT CGG TGG ATC TAA TTC CGG CGC CTA GAG AAG GAG S27-CCC-FAM AGA ATT AAG TTA CCC TAG TTA AGT TAT -(FAM) HIV Pol FOR A GAA AGG ATC ACC AGC AAT ATT CCA GTG TAG C HIV PolVif A REV A CTT TAT CTG TTT TGG TTT TAT TAA TGC TGC TAG TGC HIV PolVif B REV B aa aga ttc ATC TTA GAG CCT TTT AGA AAA CAA AAT CCA GAC ATA G HIV PolVif Sequencing GCA TTG GGA ATC ATT CAA GCA CAA CC hAID realtime FOR GGA CAG CCT CTT GAT GAA CCG β-actin realtime FOR GCC CAA CCG TGG CAC CTG ATT GCC Vλ PCR FOR TAG CAT ATG GCG GGG CCG TCA CTG ATT GCC G Vλ PCR REV GCG CAA GCT TCC CCA GCC TGA CCC CAA GTC CAA GCC TAG CCC TGC CAA GCC CAC CTG CTC CTG CTC CCC CCC CCC CCC CCC CC	HIV cDNA realtime REV	GAG TCC TGC GTC GAG AGA GC
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$\begin{array}{lll} HIV \ PolVif \ Sequencing & GCA \ TTG \ GGA \ ATC \ ATT \ CAA \ GCA \ CAA \ CC \\ hAID \ realtime \ FOR & GGA \ CAG \ CCT \ CTT \ GAT \ GAA \ CCG \ G \\ hAID \ realtime \ REV & GAC \ CCT \ TAG \ CCC \ AGC \ GGA \\ \beta-actin \ realtime \ FOR & GCC \ AAC \ CGT \ GAA \ AAG \ ATG \ ACC \ C \\ \beta-actin \ realtime \ REV & CCT \ CGT \ AGA \ TGG \ GCA \ CAG \ TGT \\ V_{\lambda} \ PCR \ FOR & TAG \ CAT \ ATG \ GCG \ GGG \ CCG \ TCA \ CTG \ ATT \ GCC \ G \\ V_{\lambda} \ PCR \ REV & GCG \ CAA \ GCT \ TCC \ CCA \ GCC \ CAA \ GTC \ CAA \ G \\ \end{array}$	HIV Pol FOR B	aa gaa ttc ATC TTA GAG CCT TTT AGA AAA CAA AAT CCA GAC ATA G
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	hAID realtime FOR	GGA CAG CCT CTT GAT GAA CCG G
β-actin realtime REVCCT CGT AGA TGG GCA CAG TGT V_{λ} PCR FORTAG CAT ATG GCG GGG CCG TCA CTG ATT GCC G V_{λ} PCR REVGCG CAA GCT TCC CCA GCC TGA CGC CAA GTC CAA G	hAID realtime REV	GAC CCT TAG CCC AGC GGA
$\begin{array}{ccc} V_{\lambda} \ PCR \ FOR & TAG \ CAT \ ATG \ GCG \ GGG \ CCG \ TCA \ CTG \ ATT \ GCC \ G \\ V_{\lambda} \ PCR \ REV & GCG \ CAA \ GCT \ TCC \ CCA \ GCC \ CAA \ GTC \ CAA \ G \\ \end{array}$	β-actin realtime FOR	GCC AAC CGT GAA AAG ATG ACC C
V _λ PCR REV GCG CAA GCT TCC CCA GCC TGA CGC CAA GTC CAA G	β-actin realtime REV	CCT CGT AGA TGG GCA CAG TGT
	V _λ PCR FOR	TAG CAT ATG GCG GGG CCG TCA CTG ATT GCC G
V _λ sequencing TTG TCT GTA AGC GGA TGC CGG	V _λ PCR REV	GCG CAA GCT TCC CCA GCC TGA CGC CAA GTC CAA G
	V_{λ} sequencing	TTG TCT GTA AGC GGA TGC CGG



Supplemental Figure S1

